from the ratio of the migration time of the actual oligomer to that of the internal standard. A linear relationship existed between the relative migration times (t') and the chain lengths of the homooligomers (n) in the size range examined, as shown in Fig. 2, according to the following equations:

$$p(dA)_n: t'(A_n) = 0.0161n + 0.7979 \quad \text{(R.S.D. 0.999\%)} \quad (1a)$$

$$p(dT)_n: t'(T_n) = 0.0205n + 0.9023 \quad \text{(R.S.D. 0.999\%)} \quad (1b)$$

$$p(dC)_n: t'(C_n) = 0.0182n + 0.7961 \quad \text{(R.S.D. 0.998\%)} \quad (1c)$$

$$p(dG)_n: t'(G_n) = 0.0071n + 0.9590 \quad \text{(R.S.D. 0.999\%)} \quad (1d)$$

where $p(dA)_n$, $p(dT)_n$, $p(dC)_n$ and $p(dG)_n$ are the individual homo-n-mers of adenylic, thymidylic, cytidylic and guanylic acid, respectively, and R.S.D. is the relative standard deviation.

The plots have similar slopes for the $p(dA)_{12-18}$, $p(dC)_{12-18}$ and $p(dT)_{12-18}$ samples (eqns. 1a–c), but a different slope for $p(dG)_{12-18}$ (eqn. 1d). This last slope is much lower, resulting in a different migration order depending on the base number for a mixture of the four homooligomers. For example, the migration order below 14 bases is A > C > G > T, between 14 and 18 bases A > G > C > T and above 18 bases G > A > C > T. This anomalous migration behavior may be due to the strong self-association tendency of guanosine under non-denaturing conditions, which migth cause conformational changes such as bending [10].

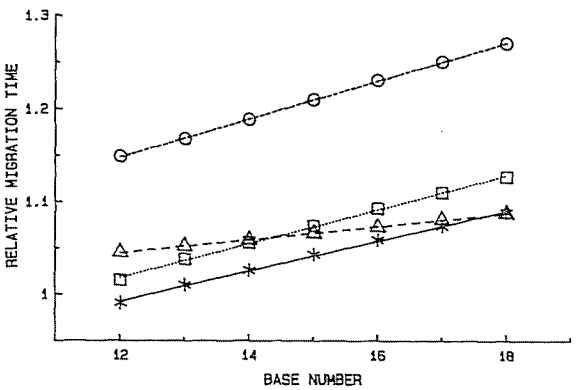

Fig. 2. Relationship between the chain length and the relative migration time of the homodeoxyribooligomer mixtures on non-denaturing polyacrylamide gel-filled capillary. Conditons as in Fig. 1. The calculation of relative migration times was based on the migration time of Orange G. * = $p(dA)_{12-18}$; ○ = $p(dT)_{12-18}$; □ = $p(dC)_{12-18}$; △ = $p(dG)_{12-18}$.

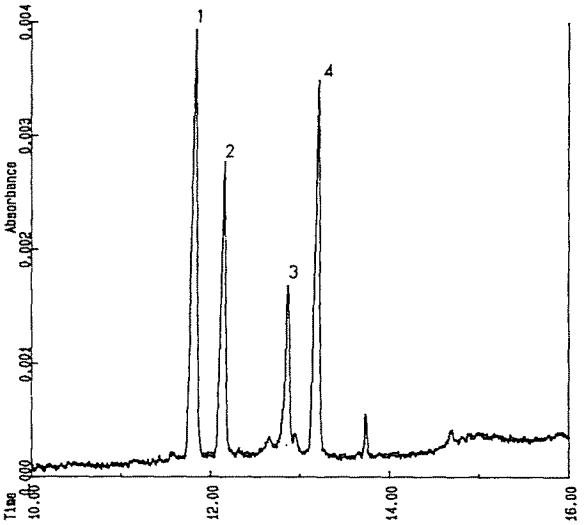

Fig. 3. Capillary polyacrylamide gel electrophoretic separation of a homodecamer mixture on non-denaturing gel. Peaks: 1 = $p(dA)_{10}$; 2 = $p(dC)_{10}$; 3 = $p(dG)_{10}$; 4 = $p(dT)_{10}$. Conditions as in Fig. 1. Migration time of Orange G (determined in the immediately following run): 12.386 min.

Also of interest in Fig. 2 is the comigration of the 14-mers of p(dC) and p(dG) and that of the 18-mers of p(dA) and p(dG). These results indicate that the migration rate of some oligomers could be relatively insensitive to a difference in base composition resulting in co-migration in non-denaturing gels.

As the base-specific retardation of oligonucleotides is an additive effect [13], migration times can be easily predicted using linear extrapolation from the relative migration times of the homooligomers. Fig. 3 shows a non-denaturing capillary gel electrophoretic separation of a mixture of four homodecamers and Table I gives the predicted and observed relative migration times of the four sample components. As can be seen in Table I, there is excellent agreement between the extrapolated and observed relative migration time values (±0.1%). The identification of the homooligomers was accomplished by spiking with the individual compounds. The small peak after peak 4 is an impurity from $p(dT)_{10}$. As Orange G migrates too close to peak 3, its migration time was determined in the immediate-

TABLE I

OBSERVED AND PREDICTED RELATIVE MIGRATION TIMES OF VARIOUS HOMO- AND HETEROOLIGODEOXYRIBONUCLEOTIDES IN NON-DENATURING POLYACRYLAMIDE CAPILLARY GEL ELECTROPHORESIS

Relative migration times were calculated using Orange G as internal standard.

Nucleotide sequence	Relative migration time		Migration order
	Observed	Calculated	
p(dA)10	0.957	0.960	1
p(dC)10	0.983	0.981	2
p(dG)10	1.039	1.032	3
p(dT)10	1.068	1.107	4
dGTTGGAGCT-G-GTGGCGTAG	1.149	1.150	1
dGTTGGAGCT-C-GTGGCGTAG	1.156	1.155	2
dGTTGGAGCT-T-GTGGCGTAG	1.160	1.161	3

ly following run, by using peak 1 [$p(dA)_{10}$] as internal standard.

Whereas simple relative migration time extrapolation is satisfactory for homooligomers, the prediction of the relative migration time of a heterooligomer (t') is improved by using the relative migration times of the four corresponding homooligomers in the following relationship:

$$t'(A_aT_tC_cG_g) = \frac{a}{n}\,t'(A_n) + \frac{t}{n}\,t'(T_n) + \frac{c}{n}\,t'(C_n) + \frac{g}{n}\,t'(G_n) \quad (2)$$

where t' is relative migration time *vs.* the internal standard, n is the oligonucleotide chain length ($n = a + t + c + g$) and a, t, c and g are the numbers of the individual bases in the oligonucleotide. The parameters $t'(A_n)$, $t'(T_n)$, $t'(C_n)$ and $t'(G_n)$ correspond to the relative migration times of the homooligo-n-mers of adenylic, thymidylic, cytidylic and guanylic acid, respectively. The most accurate calculation requires the availability of standards with chain lengths equal to the unknown. If standards are not available, the extrapolated values from linear plots such as Figs. 2 and 5 should be used. Capillary polyacrylamide gel electrophoresis of homo- and/or heterooligomers of known chain lengths and base compositions showed excellent correlation between the observed and predicted migration times (Table I).

We have also obtained (see Fig. 4) the separation of heterooligomers with the same chain lengths and similar sequences in order to emphasize the effectiveness of the mathematical prediction of the migration order. Fig. 4 shows the non-denaturing gel

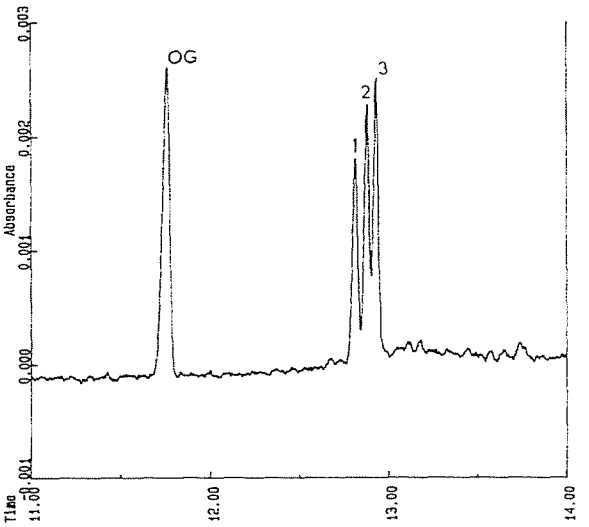

Fig. 4. Non-denaturing capillary polyacrylamide gel electrophoretic separation of a human K-ras oncogene mixture. Peaks: 1 = dGTTGGAGCT-G-GTGGCGTAG; 2 = dGTTGGAGCT-C-GTGGCGTAG; 3 = dGTTGGAGCT-T-GTGGCGTAG. Conditions as in Fig. 1.

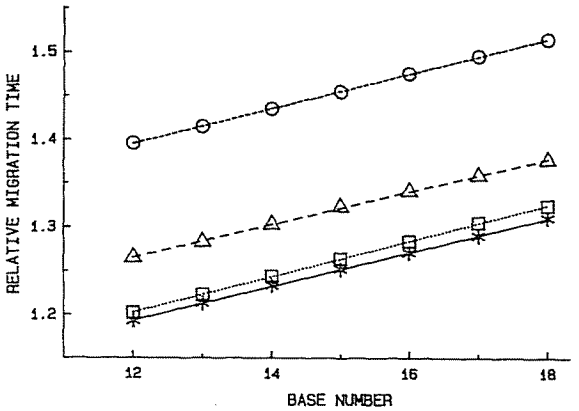

Fig. 5. Relationship between the chain length and the relative migration time of the homodeoxyribooligomer mixtures on a denaturing polyacrylamide gel-filled capillary. Conditions: isoelectrostatic (constant applied electric field), 400 V/cm; eCAP gel U100P column, effective length 40 cm, total length 47 cm; injection, 0.1 W s. The calculation of relative migration times was based on the migration time of Orange G. Symbols as in Fig. 2.

electrophoretic separation of a mixture of three human K-ras oncogenes. These oligomers have the same chain length (19-mers) and almost the same primary sequence, differing only by one base in the middle (position 10) of the chain (see primary structure in Table I). Because of the high resolving power of capillary gel electrophoresis, this method is capable of separating these closely related species. Note that the observed and predicted migration times are very similar (see Table I). The location of guanosine in the tenth position speeds up the migration rate of the heterooligomer, relative to that of cytidine or thymidine. This migration order is consistent with the data in Fig. 2. The identity of the oligomers was confirmed by spiking the mixture with the individual compounds. As no homooligo-nanodecamers were available for these experiments, the $t'(A_n)$, $t'(T_n)$, $t'(C_n)$ and $t'(G_n)$ parameters for eqn. 2 were calculated by extrapolation of the linear plots in Fig. 2.

Denaturing capillary polyacrylamide gel columns

When denaturing gels (eCAP gel U100P) were used for the separation of the same oligonucleotide mixtures, different migration properties were observed. In contrast to the behavior seen in Fig. 2, all four of the homooligomer mixtures have parallel plots of relative migration time as a function of the chain lengths, as shown in Fig. 5, according to the following equations:

$$p(dA)_n: t'(A_n) = 0.0193n + 0.9609 \quad \text{(R.S.D. 0.999\%)} \tag{3a}$$

$$p(dT)_n: t'(T_n) = 0.0202n + 1.1559 \quad \text{(R.S.D. 0.999\%)} \tag{3b}$$

$$p(dC)_n: t'(C_n) = 0.0204n + 0.9590 \quad \text{(R.S.D. 0.999\%)} \tag{3c}$$

$$p(dG)_n: t'(G_n) = 0.0188n + 1.0398 \quad \text{(R.S.D. 0.999\%)} \tag{3d}$$

TABLE II

OBSERVED AND PREDICTED RELATIVE MIGRATION TIMES OF VARIOUS HOMO- AND HETEROOLIGODEOXYRIBONUCLEOTIDES IN DENATURING POLYACRYLAMIDE CAPILLARY GEL ELECTROPHORESIS

Relative migration times were calculated using Orange G as internal standard.

Nucleotide sequence	Relative migration time		Migration order
	Observed	Calculated	
p(dA)10	1.153	1.155	1
p(dC)10	1.163	1.162	2
p(dG)10	1.237	1.233	3
p(dT)10	1.356	1.355	4
dGTTGGAGCT–G–GTGGCGTAG	1.427	1.422	2
dGTTGGAGCT–C–GTGGCGTAG	1.416	1.419	1
dGTTGGAGCT–T–GTGGCGTAG	1.430	1.429	3

The non-parallel discrepancy previously observed on non-denaturing gels does not occur in this instance, probably owing to the denaturing effect of the 7 *M* urea in the gel [14]. By means of eqn. 2, relative migration times can be calculated in a similar way as above. The relative migration data for the samples are summarized in Table II, which shows the observed and calculated migration times of the homo- and heterooligonucleotide mixtures separated on denaturing gel. Referring back to the migration behavior of the oligonucleotides on non-denaturing gels, it is important to note that the migration order has been changed among the three human K-ras oncogenes (Tables I and II). Again, this was confirmed by spiking the mixture with the individual oncogenes. As in this instance the increasing guanosine content does not have the same accelerating effect as was observed on the non-denaturing gel, the migration order is the same as that observed for the homooligomers (Table II, A > C > G > T). The accelerating effect of A- and C-rich oligomers and the retarding effect of G- and T-rich oligomers can be predicted for the chain-length range examined.

CONCLUSIONS

Investigations of the electrophoretic migration behavior of various homo-and heterooligomers of known nucleotide sequences have been presented. Using non-denaturing gels, we found that the relative migration order is not constant for homooligomers of the same chain length, but is dependent on the base number: for base numbers less than 14, it is A > C > G > T, and for base numbers larger than 18, it is G > A > C > T. This discrepancy is probably caused by the strong self-association tendency of guanosine (conformation changes such as bending). Employing denaturing gels, however, the migration order of the homooligomers is the same for the entire chain-length range examined, namely A > C > G > T. In this instance, the self-association effect of the guanosine is assumed to be negligible owing to the presence of urea, a denaturing agent, in the gel. The denaturing gel has a much higher sensitivity to guanosine content than the non-denaturing gel. There is, however, an increased ability of the non-denaturing gel to resolve A and C over the denaturing gel (compare Figs. 2 and 5). Because of the parallel slopes achieved using denaturing gels, we believe that the migration times of heterooligonucleotides are more predictable in this instance. In addition, it can be demonstrated using eqn. 2 that several combinations of sequences might be resolved in one system and not in another. Therefore, in order to increase the confidence in oligomer identity, one might need to utilize both denaturing and non-denaturing conditions.

Oligonucleotides of different sequences, but with the same chain length, are more likely to show different migration times. With respect to base composition, an equation was derived in order to predict the migration time of a known oligonucleotide sequence. Reproducibility and additivity of base-specific retardation are the basis of the calculation procedure for the relative migration times. It should be emphasized that eqn. 2 is considered to be valid only for primer-sized oligonucleotides ($n < 25$), and it should be further evaluated for longer ones.

The method opens up a new feature of capillary polyacrylamide gel electrophoresis in the identification of, and discrimination between, oligonucleotides by their mobility shift relative to an internal standard. This can be easily computed by an automated capillary electrophoretic system, such as the P/ACE 2100. A further interesting application of this equation is to calculate the migration time differences between oligonucleotides, and to design appropriate electrophoresis conditions, such as column length, necessary for their separation. This can be important for separations of oligonucleotides of the same chain length but different base composition, such as two strands of double-stranded DNA (denaturing gel), or in point mutation studies (non-denaturing gel). Whereas denaturing conditions are necessary to reduce guanosine self-association and solve compression problems, non-denaturing gels might also offer different selectivity in certain instances.

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TAB 7

CAPILLARY ELECTROPHORESIS

THEORY & PRACTICE

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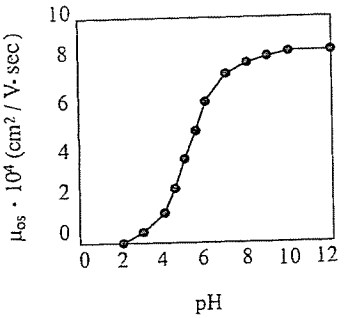

Figure 9 Dependence of electroosmotic mobility, μ_{os}, on buffer pH for the case of a fused-silica capillary that has been prewashed with 1.0 *M* NaOH. Reprinted with permission from Applied Biosystems (1990).

equation (Fahien, 1983),

$$Q = \frac{\pi \Delta P R_i^4}{8 L_{tot} \eta} \tag{50}$$

where ΔP is the pressure drop across the capillary and L_{tot} is its length. Thus, to generate a flow of $3.53 \cdot 10^{-12}$ m^3/sec would require a $\Delta P/L$ of $2.3 \cdot 10^4$ N/m^2/m or 3.4 psi/m. Thus, for this example, with regard to volumetric flow rate, an electrical potential of 30,000 V/m is comparable to a pressure gradient of 3.4 psi/m.

C. Control of Electroosmosis

For many applications it is desirable to be able to manipulate the magnitude of the electroosmotic flow in order to optimize separation performance. Many studies have been conducted describing various methods that can be used to control electroosmotic flow.

In order to control electroosmosis, it is clear from Eqs. (46) or (47) that one must control either the charge density on the capillary wall, the double-layer thickness, or the viscosity of the solution adjacent to the capillary wall. This can be clearly seen if we express Eq. (47) in terms of the double-layer thickness, κ^{-1},

$$\mu_{os} = \frac{\sigma^* \kappa^{-1}(\varepsilon, C_i)}{\eta} \tag{51}$$

where the dependence of κ^{-1} on ε and C_i has been indicated. Therefore, each of the techniques that follow acts by affecting one or the other of these parameters.

Two approaches have been used to control μ_{os} by reducing the double-layer

mail order *n* (1867) : an order for goods that is received and filled by mail

mail–order house *n* (1906) : a retail establishment whose business is conducted by mail

¹maim \'mām\ *vt* [ME *maynhen, maymen,* fr. OF *maynier*] (14c) **1** : to commit the felony of mayhem upon **2** : to mutilate, disfigure, or wound seriously — **maim·er** *n*

syn MAIM, CRIPPLE, MUTILATE, BATTER, MANGLE mean to injure so severely as to cause lasting damage. MAIM implies the loss or injury of a bodily member through violence; CRIPPLE implies the loss or serious impairment of an arm or leg; MUTILATE implies the cutting off or removal of an essential part of a person or thing thereby impairing its completeness, beauty, or function; BATTER implies a series of blows that bruise deeply, deform, or mutilate; MANGLE implies a tearing or crushing that leaves deep extensive wounds.

²maim *n* (14c) **1** *obs* : serious physical injury; *esp* : loss of a member of the body **2** *obs* : a serious loss

¹main \'mān\ *n* [in sense 1, fr. ME, fr. OE *mægen;* akin to OHG *magan* strength, OE *magan* to be able; in other senses, fr. *²main* or by shortening — more at MAY] (bef. 12c) **1** : physical strength : FORCE — used in the phrase *with might and main* **2 a** : MAINLAND **b** : HIGH SEA **3** : the chief part : essential point ⟨they are in the ~ well-trained⟩ **4** : a pipe, duct, or circuit which carries the combined flow of tributary branches of a utility system **5 a** : MAINMAST **b** : MAINSAIL

²main *adj* [ME, fr. OE *mægen-,* fr. *mægen* strength] (bef. 12c) **1** : CHIEF, PRINCIPAL ⟨the ~ drag⟩ ⟨the ~ idea⟩ ⟨her ~ man⟩ **2** : fully exerted : SHEER ⟨~ force⟩ ⟨by ~ strength⟩ **3** *obs* : of or relating to a broad expanse (as of sea) **4** : connected with or located near the mainmast or mainsail **5** : expressing the chief predication in a complex sentence ⟨the ~ clause⟩

Maine coon *n* (1935) : any of a breed of large long-haired domestic cats that have a very full tapered tail — called also *coon cat, Maine cat*

main·frame \'mān-ˌfrām\ *n* (1964) : a computer with its cabinet and internal circuits; *also* : a large computer.

main·land \'mān-ˌland, -lənd\ *n* (14c) : a continent or the main part of a continent as distinguished from an offshore island or sometimes from a cape or peninsula — **main·land·er** \-ər\ *n*

¹main·line \'mān-ˌlīn\ *vt, slang* (1938) : to take by or as if by mainlining ~ *vi, slang* : to inject a narcotic drug (as heroin) into a principal vein

²main·line \'mān-ˌlīn\ *adj* (1941) : being part of an established group; *also* : being in the mainstream

main line *n* (1841) **1** : a principal highway or railroad line **2** *slang* : a principal vein of the circulatory system

main·ly \'mān-lē\ *adv* (13c) **1** *obs* : in a forceful manner **2** : for the most part : CHIEFLY

main·mast \'mān-ˌmast, -məst\ *n* (15c) : a sailing ship's principal mast usu. second from the bow

main·sail \'mān-ˌsāl, 'mān(t)-səl\ *n* (15c) : the principal sail on the mainmast — see SAIL illustration

main·sheet \'mān-ˌshēt\ *n* (15c) : a rope by which the mainsail is trimmed and secured

main·spring \'mān-ˌsprin\ *n* (1591) **1** : the chief spring in a mechanism esp. of a watch or clock **2** : the chief or most powerful motive, agent, or cause

main·stay \-ˌstā\ *n* (15c) **1** : a ship's stay extending from the maintop forward usu. to the foot of the foremast **2** : a chief support

main stem *n* (1832) : a main trunk or channel: as **a** : the main course of a stream **b** : the main line of a railroad **c** : the main street of a city or town

¹main·stream \'mān-ˌstrēm\ *n* (1831) : a prevailing current or direction of activity or influence — **mainstream** *adj*

²main·stream \'mān-'strēm\ *vt* (1974) : to place (as a handicapped child) in regular school classes

Main Street *n* (1598) **1** : the principal street of a small town **2** *a* : the sections of a country centering about its small towns **b** : a place or environment characterized by materialistic self-complacent provin-

main·tain \mān-'tān, mən-\ *vt* [ME *mainteinen,* fr. OF *maintenir,* fr. ML *manutenēre,* fr. L *manu tenēre* to hold in the hand] (14c) **1** : to keep in an existing state (as of repair, efficiency, or validity) : preserve from failure or decline ⟨~ machinery⟩ **2** : to sustain against opposition or danger : uphold and defend ⟨~ a position⟩ **3** : to continue or persevere in : CARRY ON, KEEP UP ⟨couldn't ~ his composure⟩ **4 a** : to support or provide for : bear the expense of ⟨has a family to ~⟩ **b** : SUSTAIN ⟨enough food to ~ life⟩ **5** : to affirm in or as if in argument : ASSERT ⟨~*ed* that all men are not equal⟩ — **main·tain·abil·i·ty** \-ˌtā-nə-'bil-ət-ē\ *n* — **main·tain·able** \-'tā-nə-bəl\ *adj* — **main·tain·er** *n*

syn MAINTAIN, ASSERT, DEFEND, VINDICATE, JUSTIFY mean to uphold as true, right, just, or reasonable. MAINTAIN stresses firmness of conviction; ASSERT suggests determination to make others accept one's claim; DEFEND implies maintaining in the face of attack or criticism; VINDICATE implies successfully defending; JUSTIFY implies showing to be true, just, or valid by appeal to a standard or to precedent.

maintenir] (15c) **1** : the act of maintaining : the state of being maintained : SUPPORT **2** : something that maintains **3** : the upkeep of property or equipment **4** : an officious or unlawful intermeddling in a legal suit by assisting either party with means to carry it on

main·top \'mān-ˌtäp\ *n* (15c) : a platform about the head of the mainmast of a square-rigged ship

main–top·mast \mān-'täp-ˌmast, -məst\ *n* (15c) : a mast next above the mainmast

main yard *n* (15c) : the yard of a mainsail

mair \'mār\ *chiefly Scot var of* MORE

mai·son·ette \ˌmāz-ᵊn-'et, ˌmās-\ *n* [F *maisonnette,* fr. OF, dim. of *maison* house, fr. L *mansion-, mansio* dwelling place — more at MANSION] (1793) **1** : a small house **2** : an apartment often on two floors

maî·tre d' *or* **mai·tre d'** \ˌmā-trə-'dē, ˌme-; ˌmāt-ər-'dē, ˌmet-\ *n, pl* **maîtres d's** *or* **maitre d's** \-'dēz\ (1950) : MAÎTRE D'HÔTEL

maî·tre d'hô·tel \ˌmā-trə-(ˌ)dō-'tel, ˌme-; ˌmāt-dō-, ˌmet-\ *n, pl* **maîtres d'hôtel** *same*\ [F, lit., master of house] (1540) **1 a** : MAJORDOMO **b** : HEADWAITER **2** : a sauce of butter, parsley, salt, pepper, and lemon juice — called also *maître d'hôtel butter*

maize \'māz\ *n* [Sp *maíz,* fr. Taino *mahiz*] (1555) : INDIAN CORN

ma·ja·gua \mə-'häg-wə\ *n* [AmerSp, fr. Taino] (ca. 1903) : either of t tropical trees of the mallow family that are often considered vari forms of a single species: **a** : an irregularly spreading or shrubby (*Hibiscus tiliaceus*) that yields a light tough wood and a fibrous bas : an erect forest tree (*H. elatus*) of the West Indian uplands that yi a moderately dense timber with variegated heartwood that is used for cabinetwork and the stocks of guns

ma·jes·tic \mə-'jes-tik\ *adj* (1601) : having or exhibiting maje : STATELY *syn* see GRAND — **ma·jes·ti·cal·ly** \-ti-k(ə-)lē\ *adv*

maj·es·ty \'maj-ə-stē\ *n, pl* **-ties** [ME *maieste,* fr. MF *majesté,* fr. L *m estat-, majestas;* akin to L *major* greater] (14c) **1** : sovereign po authority, or dignity **2** — used in addressing or referring to reign sovereigns and their consorts ⟨Your *Majesty*⟩ ⟨Her *Majesty's* Gove ment⟩ **3 a** : royal bearing or aspect : GRANDEUR **b** : greatness splendor of quality or character

ma·jol·i·ca \mə-'jäl-i-kə\ *also* **ma·iol·i·ca** \-'yäl-\ *n* [It *maiolica,* fr. *Majolica* Majorca, fr. LL *Majorca*] (1555) **1** : earthenware cov with an opaque tin glaze and decorated on the glaze before firing : an Italian ware of this kind **2** : a 19th century earthenware mod in naturalistic shapes and glazed in lively colors

¹ma·jor \'mā-jər\ *adj* [ME *malour,* fr. L *major,* compar. of *mag* great, large — more at MUCH] (14c) **1** : greater in dignity, rank, portance, or interest ⟨one of the ~ poets⟩ **2** : greater in numb quantity, or extent ⟨the ~ part of his work⟩ **3** : having attained jority **4** : notable or conspicuous in effect or scope : CONSIDERABL ~ improvement⟩ **5** : involving grave risk : SERIOUS ⟨a ~ illness⟩ : of or relating to a subject of academic study chosen as a field of cialization **7 a** : having half steps between the third and fourth the seventh and eighth degrees ⟨~ scale⟩ **b** : based on a major ⟨~ key⟩ **c** : equivalent to the distance between the keynote and other tone (except the fourth and fifth) of a major scale ⟨~ third⟩ : containing a major third ⟨~ triad⟩

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ma·jor-do·mo \ˌmā-jər-'dō-(ˌ)mō\ *n, pl* **-mos** [Sp *mayordomo* or ob *maiordomo,* fr. ML *major domus,* lit., chief of the house] (1589) head steward of a large household (as a palace) **2** : BUTLER, STEW **3** : a person who speaks, makes arrangements, or takes charge another

majorette *n* (1940) : DRUM MAJORETTE 2

major general *n* [F *major général,* fr. *major,* n. + *général,* adj., gen (1642) : a commissioned officer in the army, air force, or marine c who ranks above a brigadier general and whose insignia is two stars

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ma·jus·cule \'maj-əs-ˌkyü(ə)l, mə-'jəs-\ *n* [F, fr. L *majusculus* large, dim. of *major*] (1727) : a large letter (as a capital) — **ma·jus** lar \mə-'jəs-kyə-lər\ *adj* — **majuscule** *adj*

mak·able *or* **make·able** \'mā-kə-bəl\ *adj* (15c) : capable of being ma

mak·er \'mā-kər, 'māk-\ *n* [ME *maker*] *chiefly Scot* (14c) : POET

¹make \'māk\ *vb* **made** \'mād\; **mak·ing** [ME *maken,* fr. OE *m* akin to OHG *mahhōn* to prepare, make, OSlav *mazati* to anoi (bef. 12c) **1** *a obs* : BEHAVE, ACT **b** : to seem to begin (an a ⟨*made* to go⟩ **2 a** : to cause to happen to or be experienced by one ⟨*made* trouble for us⟩ **b** : to cause to exist, occur, or appear ATE ⟨~ a disturbance⟩ **c** : to favor the growth or occurrence of

B. Relationship between Mobility and Molecular Size

The way in which the frictional coefficient, f, is related to the size of a molecule depends on what model is used to describe the conformation of the molecule and on the nature of the solvent. In this section we discuss the case of a solute migrating through free solution.

If, in free solution, the migrating molecule is modeled as a solid sphere, the relationship between the translational frictional coefficient and molecular size would be straightforward. From Stokes' law we know that

$$f = 6\pi\eta R \tag{7}$$

Furthermore, for a solid sphere, the radius and the mass, m, are related by

$$m = \rho_p\left(\frac{4}{3}\pi R^3\right) \tag{8}$$

where ρ_p is the density of the particle. Thus,

$$R \sim m^{1/3} \tag{9}$$

where the "~" symbol indicates proportionality. Therefore, from Eqs. (6) and (9)

$$f \sim m^{1/3} \tag{10}$$

If, rather than being a solid sphere, the solute behaves as a loose coil or a rod, we can no longer use Eq. (8) to directly relate the molecular mass to an apparent hydrodynamic radius and thus to a value for f. However, the relationship between mass and the translational friction coefficient has been established for a number of practically important molecular conformations. Some of these are given in Table I. Table I clearly shows that the way in which electrophoretic mobility in free solution is related to molecular size is strongly dependent on the model chosen to represent the conformation of the solute.

Table I Proportionality Relationship between Frictional Coefficient and Molecular Weight[a]

Molecular model	Proportionality relationship
Solid sphere	$f \sim (MW)^{1/3}$
Random coil — unperturbed chain	$f \sim (MW)^{0.5 \text{ to } 0.6}$
Long rod	$f \sim (MW)^{0.8}$
Wide thin disk	$f \sim (MW)^{2/3}$
Free-draining coil	$f \sim (MW)^{1.0}$

[a] Reproduced with permission from Cantor and Schimmel (1980).

As it turns out, for many practically important applications, DNA and sodium dodecyl sulfate (SDS)-treated protein applications in particular, separations based solely on differences in free-solution electrophoretic mobilities are not possible. This is a direct result of the relationship between f and the molecular size characteristic of these solutes. This can be demonstrated through a simple argument, using DNA as an example.

The structure of the DNA molecule is such that the total net charge on the molecule is directly proportional to its size; i.e., approximately two charges per base pair. Thus,

$$q \sim N \tag{11}$$

where N is the number of units (base pairs) in the DNA chain. In addition, since DNA exists in solution as a free-draining coil, it can be seen from Table I that

$$f \sim N \tag{12}$$

(A free-draining coil is one in which each of the units of the chain contributes equally to the overall drag of the chain.) Finally, by combining Eqs. (11) and (12) with the definition for electrophoretic mobility, Eq. (6), it can be seen that μ is no longer a function of molecular size, i.e.,

$$\mu = \frac{q}{f} \sim \frac{N}{N} = N^0 \tag{13}$$

where N^0 indicates that μ is constant with respect to changes in N. Thus, because both the charge and the frictional drag are proportional to molecular size, free-solution electrophoretic separations of DNA are impossible. This argument also applies to the case of SDS proteins in which charge is also directly proportional to N, and the polymer behaves as a free-draining coil. Therefore, in order to perform a separation of DNA fragments, or of any molecule with a constant ratio of net charge to translational friction coefficient, one must exploit an alternate separation mechanism. If, instead of allowing the DNA molecule to migrate in free solution, one forces it to travel through a porous polymer network, one can impart a size dependence to the electrophoretic mobility. The migration of chainlike molecules through a polymer network will be discussed in Chapter 8.

C. Effect of Buffer Ions on the Effective Charge and Mobility

1. Charge Screening

In an electrolyte solution, when a charged solute is moving, the effective charge of the solute, q, is less than the total charge, owing to the screening influence of the counterions in solution. This shielding is attributable to the presence of counterions, located within a stagnant layer immediately adjacent to the surface of the solute (see Fig. 1). The stagnant layer is caused by the viscous force acting between the solvent and the surface of the solute. The interface between the stagnant layer and the surrounding solution is called the surface of shear. The exact position of the surface of shear is not

TAB 8

WEBSTER'S Ninth New Collegiate Dictionary

Library of Congress Cataloging in Publication Data
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mail order *n* (1867) : an order for goods that is received and filled by mail

mail–order house *n* (1906) : a retail establishment whose business is conducted by mail

¹maim \'mām\ *vt* [ME *maynhen, maymen,* fr. OF *maynier*] (14c) **1** : to commit the felony of mayhem upon **2** : to mutilate, disfigure, or wound seriously — **maim·er** *n*

syn MAIM, CRIPPLE, MUTILATE, BATTER, MANGLE mean to injure so severely as to cause lasting damage. MAIM implies the loss or injury of a bodily member through violence; CRIPPLE implies the loss or serious impairment of an arm or leg; MUTILATE implies the cutting off or removal of an essential part of a person or thing thereby impairing its completeness, beauty, or function; BATTER implies a series of blows that bruise deeply, deform, or mutilate; MANGLE implies a tearing or crushing that leaves deep extensive wounds.

²maim *n* (14c) **1** *obs* : serious physical injury; *esp* : loss of a member of the body **2** *obs* : a serious loss

¹main \'mān\ *n* [in sense 1, fr. ME, fr. OE *mægen;* akin to OHG *magan* strength, OE *magan* to be able; in other senses, fr. *²main* or by shortening — more at MAY] (bef. 12c) **1** : physical strength : FORCE — used in the phrase *with might and main* **2 a** : MAINLAND **b** : HIGH SEA **3** : the chief part : essential point ⟨they are in the ~ well-trained⟩ **4** : a pipe, duct, or circuit which carries the combined flow of tributary branches of a utility system **5 a** : MAINMAST **b** : MAINSAIL

²main *adj* [ME, fr. OE *mægen-,* fr. *mægen* strength] (bef. 12c) **1** : CHIEF, PRINCIPAL ⟨the ~ drag⟩ ⟨the ~ idea⟩ ⟨her ~ man⟩ **2** : fully exerted : SHEER ⟨~ force⟩ ⟨by ~ strength⟩ **3** *obs* : of or relating to a broad expanse (as of sea) **4** : connected with or located near the mainmast or mainsail **5** : expressing the chief predication in a complex sentence ⟨the ~ clause⟩

Maine coon *n* (1935) : any of a breed of large long-haired domestic cats that have a very full tapered tail — called also *coon cat, Maine cat*

main·frame \'mān-ˌfrām\ *n* (1964) : a computer with its cabinet and internal circuits; *also* : a large computer

main·land \'mān-ˌland, -lənd\ *n* (14c) : a continent or the main part of a continent as distinguished from an offshore island or sometimes from a cape or peninsula — **main·land·er** \-ər\ *n*

¹main·line \'mān-ˌlīn\ *vt, slang* (1938) : to take by or as if by mainlining ~ *vi, slang* : to inject a narcotic drug (as heroin) into a principal vein

²main·line \'mān-ˌlīn\ *adj* (1941) : being part of an established group; *also* : being in the mainstream

main line *n* (1841) **1** : a principal highway or railroad line **2** *slang* : a principal vein of the circulatory system

main·ly \'mān-lē\ *adv* (13c) **1** *obs* : in a forceful manner **2** : for the most part : CHIEFLY

main·mast \'mān-ˌmast, -məst\ *n* (15c) : a sailing ship's principal mast usu. second from the bow

main·sail \'mān-ˌsāl, 'mān(t)-səl\ *n* (15c) : the principal sail on the mainmast — see SAIL illustration

main·sheet \'mān-ˌshēt\ *n* (15c) : a rope by which the mainsail is trimmed and secured

main·spring \'mān-ˌspriŋ\ *n* (1591) **1** : the chief spring in a mechanism esp. of a watch or clock **2** : the chief or most powerful motive, agent, or cause

main·stay \-ˌstā\ *n* (15c) **1** : a ship's stay extending from the maintop forward usu. to the foot of the foremast **2** : a chief support

main stem *n* (1832) : a main trunk or channel: as **a** : the main course of a stream **b** : the main line of a railroad **c** : the main street of a city or town

¹main·stream \'mān-ˌstrēm\ *n* (1831) : a prevailing current or direction of activity or influence — **mainstream** *adj*

²main·stream \'mān-'strēm\ *vt* (1974) : to place (as a handicapped child) in regular school classes

Main Street *n* (1598) **1** : the principal street of a small town **2** a : the sections of a country centering about its small towns **b** : a place or environment characterized by materialistic self-complacent provincialism — **Main Street·er** \'mān-ˌstrēt-ər\ *n*

main·tain \mān-'tān, mən-\ *vt* [ME *mainteinen,* fr. OF *maintenir,* fr. ML *manutenēre,* fr. L *manu tenēre* to hold in the hand] (14c) **1** : to keep in an existing state (as of repair, efficiency, or validity) : preserve from failure or decline ⟨~ machinery⟩ **2** : to sustain against opposition or danger : uphold and defend ⟨~ a position⟩ **3** : to continue or persevere in : CARRY ON, KEEP UP ⟨couldn't ~ his composure⟩ **4 a** : to support or provide for : bear the expense of ⟨has a family to ~⟩ **b** : SUSTAIN ⟨enough food to ~ life⟩ **5** : to affirm in or as if in argument : ASSERT ⟨~*ed* that all men are not equal⟩ — **main·tain·abil·i·ty** \-ˌtā-nə-'bil-ət-ē\ *n* — **main·tain·able** \-'tā-nə-bəl\ *adj* — **main·tain·er** *n*

syn MAINTAIN, ASSERT, DEFEND, VINDICATE, JUSTIFY mean to uphold as true, right, just, or reasonable. MAINTAIN stresses firmness of conviction; ASSERT suggests determination to make others accept one's claim; DEFEND implies maintaining in the face of attack or criticism; VINDICATE implies successfully defending; JUSTIFY implies showing to be true, just, or valid by appeal to a standard or to precedent.

main·te·nance \'mānt-nən(t)s, -ᵊn-ən(t)s\ *n* [ME, fr. MF, fr. OF, fr. *maintenir*] (15c) **1** : the act of maintaining : the state of being maintained : SUPPORT **2** : something that maintains **3** : the upkeep of property or equipment **4** : an officious or unlawful intermeddling in a legal suit by assisting either party with means to carry it on

main·top \'mān-ˌtäp\ *n* (15c) : a platform about the head of the mainmast of a square-rigged ship

main–top·mast \mān-'täp-ˌmast, -məst\ *n* (15c) : a mast next above the mainmast

main yard *n* (15c) : the yard of a mainsail

mair \'mār\ *chiefly Scot var of* MORE

mai·son·ette \ˌmāz-ᵊn-'et, ˌmās-\ *n* [F *maisonnette,* fr. OF, dim. of *maison* house, fr. L *mansion-, mansio* dwelling place — more at MANSION] (1793) **1** : a small house **2** : an apartment often on two floors

mai·tre d' *or* mai·tre d' \ˌmā-trə-'dē, ˌme-; ˌmāt-ər-'dē, ˌmet-\ *n, pl* **maître d's** *or* **maitre d's** \-'dēz\ (1950) : MAÎTRE D'HÔTEL

maî·tre d'hô·tel \ˌmā-trə-(ˌ)dō-'tel, ˌme-; ˌmāt-dō-, ˌmet-\ *n, pl* **maîtres d'hôtel** *same*\ [F, lit., master of house] (1540) **1 a** : MAJORDOMO **b** : HEADWAITER **2** : a sauce of butter, parsley, salt, pepper, and lemon juice — called also *maître d'hôtel butter*

maize \'māz\ *n* [Sp *maíz,* fr. Taino *mahiz*] (1555) : INDIAN CORN

ma·ja·gua \mə-'häg-wə\ *n* [AmerSp, fr. Taino] (ca. 1903) : either of two tropical trees of the mallow family that are often considered varieties forms of a single species: **a** : an irregularly spreading or shrubby tree (*Hibiscus tilaceus*) that yields a light tough wood and a fibrous bast **b** : an erect forest tree (*H. elatus*) of the West Indian uplands that yields a moderately dense timber with variegated heartwood that is used for cabinetwork and the stocks of guns

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CERTIFICATE OF SERVICE

I, Karen E. Keller, Esquire, hereby certify that on January 20, 2006, I caused to be electronically filed a true and correct copy of the foregoing document with the Clerk of the Court using CM/ECF, which will send notification that such filing is available for viewing and downloading to the following counsel of record:

Frederick L. Cottrell, III, Esquire
Steve Fineman, Esquire
RICHARDS, LAYTON & FINGER
One Rodney Square
Wilmington, Delaware 19801

I further certify that on January 20, 2006, I caused a copy of the foregoing document to be served by hand delivery on the above-listed counsel of record and on the following non-registered participants in the manner indicated:

BY FEDERAL EXPRESS

Wayne L. Stoner, Esquire
Wilmer Cutler Pickering Hale and Dorr LLP
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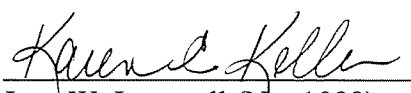

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